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A STUDY OF THE RATE OF THE INTERSTITIAL GROWTH OF THE PERSISTENT TEETH OF THE ALBINO RAT, AS SHOWN BY VITAL DYES

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I. INTRODUCTION

In a recent series of studies, with vital dyes, on the developing dental tissues of the albino rat (3), I constantly noticed, in the dentine of these animals, certain colored lines that corresponded in tint to the dyes injected and to the intervals between the injections. The thought at once arose that possibly here was a reliable method of measuring the interstitial growth of the incisor or persistent teeth. With this end in view, I have examined over one hundred jaws of rats with the teeth *in situ*, hoping thereby to arrive at definite conclusions. The results of this work are recorded on the following pages.

II. EXPERIMENTAL PROCEDURE

Technique

The animals upon which these investigations are based were injected, from time to time, by Prof. H. M. Evans, his assistants and

myself, for the primary purpose of studying certain cells, tissues, and organs, and the effects of vital dyes upon them. After autopsy, the heads of these animals were immediately prepared by me for future study and examination.

Each head, in a glass container and covered with warm 10 per cent solution of formol, was placed in an electric oven at a temperature of 35°C. for from three days to one week. Upon removal from the oven, both jaws were dissected from the head, each divided on the median line, the parts again placed in warm 10 per cent aqueous solution of formol, and returned to the oven at the previous temperature. Later, the parts were passed through the alcohols, from 40 per cent to absolute, increasing 5 per cent each successive twelve hours, and then placed for from one to three weeks in benzo-dammar, at room temperature. This method of preparation was worked out after many discouraging failures with zylol-balsam. The special advantage of benzo-dammar over zylol-balsam is the greater rapidity with which it hardens and the lesser liability of gumming the wheels in grinding.

When the specimens were thoroughly impregnated with dammar, they were transferred to the disks of a grinding machine, cemented in place with thicker benzo-dammar, again placed in an electric oven, this time at a temperature of 50°C., and allowed to remain for two or three days, or until the dammar was sufficiently hardened to prevent the specimen from being torn from the disk in grinding.

Dyes employed

Various dyes have been employed, including violet, purple, blue, pink, red and brown. It was soon found, however, that the only colors of real value for my purposes were the violet, purple, and blue. From this time on, therefore, the heads of only the rats that had been injected with the violet, purple, or blue dyes, were prepared by me for this study. These dyes stained the pulp, the odontoblasts, the pericementum, and cementum, and formed certain narrow lines in the dentine. These lines supposedly corresponded to the tint of the dye employed, the number of the injections, and the intervals between the injections. Fearing, however, that I might be unconsciously influenced in my deductions that the dyes produced the lines

in the dentine and also that the distances between the color lines were determined by the lengths of the periods between the injections, I requested Professor Evans and his assistants to keep from me all knowledge of the color of the dyes injected, and the number and lengths of the periods between the injections, until after my examination of each case was completed. In comparing our records, it was found that my suppositions in relation to the formation of the color lines, and the distances between them, were correct.

All solutions of the dyes, and their injections, were made under the strictest aseptic precautions. No animal at any time during the experiments was made seriously ill from sepsis or any other cause. Occasionally a slight indisposition was manifested soon after an injection, but this was nothing more than a degree of uneasiness that passed off in from two to five minutes. In a few instances the animal-house records show that an animal suffered from some affection of the lungs or bowels, but this was no more common among the rats under treatment than among those of the colony that had never been under treatment. In one instance a rat under treatment was found dead in the morning. It was immediately autopsied, but no cause of death could be discovered. Caged animals when kept in groups are inclined to huddle together, and this animal might have been smothered.

In every case where a violet, purple, or blue dye had been employed in sufficient strength, the dye was in evidence in a colored line in the dentine, that of the most recent injection being near the pulp and the intervals between the injections, if more than one day apart, were well marked in this way. The pink, red and brown dyes, even when administered in fairly large doses did not give sufficient contrast to be readily observed through the microscope. (See page 251 for exceptional results with alizarine.)

The lower powers of the microscope were most serviceable for observing the *color lines* and for counting them. The color lines always showed most prominently, in longitudinal sections, in that portion of the dentine that lies between the pulp and the enamel. For some reason the lines are not as distinct between the pulp and the cementum.

Dosage

The doses employed ranged between 1 cc. and 6 cc. of solutions varying in strength from 0.1 per cent to 5 per cent, most commonly from 0.5 per cent to 1.0 per cent. A solution of a lower per cent than these was too weak to leave the distinct characteristic markings in the dentine, while solutions above 1 per cent seemed to give no better results than 1 cc. of a 1 per cent solution. The intervals between the injections were purposely arranged at one, two, three, four, and five days; the lines and distances between them observed; and the slides carefully numbered, marked and preserved.

The dyes that seemed to give the best results were naphthamine brilliant blue and trypan blue, employed in 1 per cent aqueous solution and in 1 cc. doses. The pink, red and brown dyes were not satisfactory, as there was not sufficient contrast to give pronounced color lines in the dentine. The red dyes showed best in the pulp and the blood-vessels, but they did not stain the developing cells and tissues as intensely as did the violet, purple, and blue dyes. The latter dyes always stained the pulp and odontoblasts, and left a narrow line of color in the dentine near the pulp. The position of this line depended upon the length of the interval of time betweeen the last injection and the arranged demise of the animal. The ameloblasts and the secreting papillae of Williams were also stained, but this color was deposited from the capillary blood vessels in the inner tunic of the enamel organ, and not through the blood vessels of the pulp.

III. EXPERIMENTAL FINDINGS

Incremental lines

The incremental or growth lines are readily seen in the teeth of all the smaller animals of the class Mammalia, with the aid of the lower powers of the microscope; while in the tusks of the elephant, and in the persistent cuspids of the hippopotamus, they can be seen with the unaided eye. In certain of the smaller rodents, like mice and rats, these lines are also in evidence under the somewhat higher powers of the microscope.

The incremental lines, or layers, are arranged in concentric form. They appear in longitudinal section in the form of superposed cones or one lying within the other (*fig. 1*). In transverse section they are seen in concentric rings, arranged one within the other, like the layers of an onion (*fig. 2*). This effect is more beautifully brought out by the injection of the vital azo dyes in the rat. *Fig. 1* was drawn from the tip of a tusk of an old elephant, cut in longitudinal sections, and shows considerable wear. *Fig. 2* was drawn from a transverse section of the same tusk, cut at the distal end of the pulp-chamber. These sections are introduced to illustrate the concentric growth of the teeth. The dentine of the teeth of all the Mammalia is formed in the same manner, layer within layer, beginning at the outer periphery of the pulp and progressing concentrically until the tooth is completed.

The above described arrangement of the laminae is brought about through the functional activity of the odontoblasts, which are generally regarded as responsible for the formation and calcification of the dentine.

The pulp is completely covered in all directions except at its base—this applies particularly to the persistent teeth—with a layer of odontoblasts, the "membrana eboris" of Kölliker, or the odontoblastic layer of Waldyer. The pulp constantly recedes (grows smaller) as the concentric laminae are formed, until the dentine is completed according to the plan of nature, in all but the persistent teeth, where it continues to form during the entire life of the animal.

Growth of the persistent teeth

In the development of the persistent teeth of the rat, there are *three* forms of growth, namely, interstitial, extrusive, and curvative. The *first* deals with the formation of the laminae or concentric layers of the dentine; the *second*, with the extrusion or pushing out of the tooth; the *third*, with the curving of the tooth. The latter condition is doubtless due to two causes: one, the more rapid growth of the enamel side of these teeth, as compared with the cementum side; the other, to the pressure of the lips upon the labial aspect, which would have a tendency to curve them toward the palate above and

the tongue below. Reference has already been made to the well-known fact that the enamel of the persistent teeth of the rat is confined to the labial aspect; and, as these teeth normally always curve in a lingual direction, it is fair to presume that this curving is brought about by the causes just indicated. To determine whether the labial aspect (enamel side) of the tooth grows more rapidly than the lingual (cementum side), certain experiments were instituted, the details of which appear in the concluding section.

In the light of the experiments recorded in the earlier portion of this report, I was inclined to the belief that the distinctness of the incremental lines, as seen in the teeth of the Mammalia, is more or less under the influence of the diet or the coloring matter contained therein. With this idea in mind, I submitted two litters of young albino rats, six in each litter aged eight weeks, to the following experiments. Two of each litter were kept separate as controls and fed a diet having no added coloring matter, namely, stale white bread soaked in fresh sweet milk. Four of one litter were fed the same diet, to which was added each fourth day 4 cc. of 1 per cent solution of alizarine sodium sulphonate in distilled water—1 cc. to each rat. The latter four rats were always separated individually until they had eaten the food to which the pigment had been added. Four of the second litter were each injected intraperitoneally, every fourth day, with 1 cc. of 1 per cent aqueous solution of alizarine sodium sulphonate and fed at all times the normal diet. Fresh water was supplied in abundance to both groups, as the alizarine seemed to make them thirsty.

These later experiments were begun when the animals were about sixty days old, the object being to ascertain whether the red color of alizarine would be shown in the dentine by colored lines, at definite distances apart, in the animals on the diet containing the dye and also in those that had been injected intraperitoneally with the same dye, in the same amount and at the same intervals.

Rate of interstitial growth

It will be recalled that in an earlier part of this report attention was invited to the fact that the violet, purple and blue dyes produced

certain well marked lines in the dentine of the same tint as the dye, and that the distances between these lines corresponded to the lengths of the intervals between the injections. These injections, it was stated, were later purposely arranged with intervals of one, two three, four, and five days, the object being to ascertain whether the growth of the dentine was responsible for the differences in the distances between the lines.

The lines do not always show with the same distinctness. This variation depends largely upon the thickness of the section under examination. The best results were obtained with slides bearing ground sections that were from twelve to fifteen microns thick. Sections that are thinner or thicker than this do not give satisfactory results. In the thinner sections the light dissipates the color; in the thicker ones, the light fails properly to penetrate the sections and the lines are obscured or confused.

Fig. 3, drawn from a slide made of the right lower jaw, with the incisor *in situ* of rat "L^2" (no. 204 of Dr. Long's series), is introduced to show the appearance of the section under a low power of the microscope.[1] The lower incisor teeth have a slightly lateral curvature, hence the appearance of the specimen—narrower in its center than at its ends. *Fig. 4* is drawn from a slide of the superior incisor of rat no. 1855 (of the author's series), the low power showing lines in the dentine.

Upon measuring the distances between the lines in the various specimens, with the stage micromillimeter divided into hundredths, it was found that the average daily interstitial growth of dentine in the persistent teeth of the rat, as indicated by these distances, was about 0.01 mm. Absolute accuracy in measurement was impossible, but the results obtained were so uniform that they were taken as approximately correct.

The following tables (*1–9*) are presented in corroboration of the above statements.

[1] See page 7 and *fig. 3*, in an earlier paper in this relation (*Journal of Dental Research*, 1920, ii), for additional facts about the treatment, and the condition of the teeth, of this rat.

TABLE 1

Rat "L²" (no. 204, Dr. Long's series); born October 19, 1916; killed November 11, 1916.
Dye: Dianil blue 2R; aqueous solution, 1 per cent.
Injections: Intraperitoneal. Eleven injections; volume, 20 cc.
Right lower jaw. Incisor *in situ*. Longitudinal section.

DATE	AMOUNT INJECTED	INTERVAL	MEASUREMENT
	cc.	*days*	*mm.*
October 22	1		
October 24	2	2	0.02
October 26	2	2	0.02
October 28	2	2	0.02
October 30	2	2	0.02
November 1	5	2	0.02
November 3	1	2	0.02
November 6	1	3	0.03
November 7	1	1	0.01
November 8	1	1	0.01
November 9	1	1	0.01
November 10	1	1	0.01
November 11. Animal killed with chloroform.			

TABLE 2

Rat no. 1,855 (author's series); full-grown animal.
Dye: Naphthamine brilliant blue 2R; aqueous solution, 1 per cent.
Injections: Intraperitoneal. Nine injections; volume, 36 cc.
Right superior jaw. Incisor *in situ*. Longitudinal section.

DATE	AMOUNT INJECTED	INTERVAL	MEASUREMENT
	cc.	*days*	*mm.*
April 30	4		
May 3	4	3	0.03
May 7	4	4	0.04
May 10	4	3	0.03
May 15	4	5	0.05
May 17	4	2	0.02
May 19	4	2	0.02
May 22	4	3	0.03
May 24	4	2	0.02
May 26. Slightly ill with peritonitis. Not injected.			
May 28. Animal killed with chloroform.			

Seven lines show distinctly, two indistinctly. Last two injections, May 22 and 24; at three and two day intervals the lines do not show distinctly, although they are faintly in evidence in the forming dentine. Pulp is stained blue. Ameloblasts and forming enamel rods are stained blue.

TABLE 3

Rat no. 9 (Dr. K. Scott's series); full-grown animal.
Dye: Trypan blue; aqueous solution, 1 per cent.
Injections: Intraperitoneal. Five injections; volume, 10 cc.
Left lower jaw. Incisor *in situ*. Longitudinal section.

DATE	AMOUNT INJECTED	INTERVAL	MEASUREMENT
	cc.	*days*	*mm.*
January 27	2		
January 29	2	2	0.02
February 1	2	3	0.03
February 4	2	3	0.03
February 6	2	2	0.02
February 7. Animal killed with chloroform.			

Animal stained intensely blue. Four blue lines show at the base of the pulp.

TABLE 4

Rat no. 9 (Dr. K. Scott's series); full-grown animal.
Dye: Trypan blue; aqueous solution, 1 per cent.
Injections: Intraperitoneal. Five injections; volume, 10 cc.
Right lower jaw. Incisor *in situ*. Transverse section.

DATE	AMOUNT INJECTED	INTERVAL	MEASUREMENT
	cc.	*days*	*mm.*
January 27	2		
January 29	2	2	0.02
February 1	2	3	0.03
February 4	2	3	0.03
February 6	2	2	0.02
February 7. Animal killed with chloroform.			

Specimen under oc. no. 4, obj. no. 5, shows four concentric blue lines. See *fig. 5*. Two other serial sections show the same.

TABLE 5

Rat no. 366 (Dr. K. Scott's series); full-grown animal.
Dye: Trypan blue; aqueous solution, 1 per cent.
Injections: Intraperitoneal. Six injections; volume, 3 cc.
Left lower jaw. Incisor *in situ*. Longitudinal section.

DATE	AMOUNT INJECTED	INTERVAL	MEASUREMENT
	cc.	*days*	*mm.*
February 11	0.5		
February 14	0.5	3	0.03
February 17	0.5	3	0.03
February 20	0.5	3	0.03
February 23	0.5	3	0.03
February 26	0.5	3	0.03
February 27. Animal killed with chloroform.			

Animal stained deep blue. Connective-tissue structures stained deep blue. Epithelial structures a dull blue or, more nearly, a dull purple. Lines in the dentine not strongly marked and show best upon the enamel side of the pulp. Lines are so faint that it is difficult to count them and measure the distances between them. Probably the solution was too weak to give good results in this respect.

TABLE 6

Rat no. 399 (Dr. K .Scott's series); full-grown animal.
Dye: Trisulphon Blau R; aqueous solution, 0.5 per cent.
Injections: Intraperitoneal. Seven injections; volume, 14 cc.
Right inferior jaw. Incisor *in situ*. Longitudinal section.

DATE	AMOUNT INJECTED	INTERVAL	MEASUREMENT
	cc.	*days*	*mm.*
February 27	2		
February 28	2	1	0.01
March 1	2	1	0.01
March 2	2	1	0.01
March 3	2	1	0.01
March 7	2	4	0.04
March 10	2	3	0.03
March 11. Animal killed with chloroform.			

Connective-tissue structures stained blue. Epithelial tissue and ameloblasts stained red.

TABLE 7

Rat no. 399 (Dr. K. Scott's series); full-grown animal.
Dye: Trisulphon Blau R; aqueous solution, 0.5 per cent.
Injections: Intraperitoneal. Seven injections; volume, 14 cc.
Left superior jaw. Incisor *in situ*. Longitudinal section.

DATE	AMOUNT INJECTED	INTERVAL	MEASUREMENT
	cc.	*days*	*mm.*
February 27	2		
February 28	2	1	0.01
March 1	2	1	0.01
March 2	2	1	0.01
March 3	2	1	0.01
March 7	2	4	0.04
March 10	2	3	0.03
March 11. Animal killed with chloroform.			

Results were similar to those indicated in *table 6*.

TABLE 8

Rat no. 441 (Dr. K. Scott's series); full-grown animal.
Dye: T—1,846; aqueous solution, 0.5 per cent.
Injections: Intraperitoneal. Six injections; volume, 12 cc.
Right inferior jaw. Incisor *in situ*. Longitudinal section.

DATE	AMOUNT INJECTED	INTERVAL	MEASUREMENT
	cc.	*days*	*mm.*
February 27	2		
February 28	2	1	0.01
March 1	2	1	0.01
March 2	2	1	0.01
March 3	2	1	0.01
March 5	2	2	0.02
March 5. Animal killed with chloroform.			

Lines very faint; too faint to be counted with certainty. Section rather thick.

TABLE 9

Rat no. 441 (Dr. K. Scott's series); full-grown animal.
Dye: T—1,846; aqueous solution, 0.5 per cent.
Injections: Intraperitoneal. Six injections; volume, 12 cc.
Left inferior jaw. Incisor *in situ*. Transverse section.

DATE	AMOUNT INJECTED	INTERVAL	MEASUREMENT
	cc.	*days*	mm.
February 27	2		
February 28	2	1	0.01
March 1	2	1	0.01
March 2	2	1	0.01
March 3	2	1	0.01
March 5	2	2	0.02
March 5. Animal killed with chloroform.			

Specimen shows five faint blue lines, last one at border of the pulp. Incisor *in situ* in right superior jaw spoiled in grinding.

Results of experiments with alizarine

In the experiments with alizarine sodium sulphonate, in 1 per cent solution in distilled water and in 1 cc. doses every fourth day until five doses had been given, it was found that the bones of each of the animals that received the dye mixed with its food were only faintly tinged with the dye, the color being a light pink; while in those that received the dye by injection intraperitoneally in the same amount and at the same intervals, the bones were tinged a rose red. This applied to all the bones of the skeleton, including the maxillary bones.

In an earlier portion of this report, I invited attention to the fact that the vital ***pink***, ***red***, and ***brown*** dyes were not satisfactory for staining the dentine—that they did not leave definite lines in this tissue, as did the violet, purple, and blue dyes. Alizarine is a ***red*** dye and, in the animals to which it had been given by ***mouth***, as just stated, it showed no other effect than that of staining slightly red the vessels of the pulp, also the forming enamel, particularly the *ameloblasts*. No lines were found in the dentine. In the animals injected ***intraperitoneally*** with the same amount of the dye and at the same intervals, however, definite red lines were formed in the dentine; and the distances between these lines were exactly

equal to those for the animals that had been injected with the violet, purple, and blue dyes, namely, 0.01 mm. per day. In these cases the injection intervals were four days, and the distances between the lines were 0.04 mm. each. *Fig. 6* shows one of the sections of the inferior jaw, the incisor *in situ* with five color lines corresponding to the number of injections and the four-day intervals between the injections.

It is possible that by giving animals larger doses by *mouth*, a more decided coloration might be developed in this way in the bones and also as definite lines in the dentine. The results of the experiments prove, however, that the injection method is superior to that of giving the dye by mouth, for pronounced results were obtained by injection, while by giving the same amount by mouth, practically no decided results were obtained. Another advantage of injection over administration by mouth was the fact that the former caused no special unpleasant after-effects on the animals, while oral administration of the dye, even in the small doses given, caused the animals to refuse food afterwards, sometimes for nearly a day.

The *control* animals gained in weight steadily during the period of the experiment. The injected animals also steadily gained in weight, but to a less degree; while those that were given the dye by mouth gained very little. The oral administration of the dye after the second time seemed to make the animals slightly ill, and caused them to take but little food.

Extrusive growth and curvative growth

To ascertain the extent of the *extrusive* growth of the persistent teeth of the rat, notches were filed in the enamel of the incisor teeth, superior and inferior, at the point or margin of the interproximal papillae of the gingivus. At the end of one week the animals were killed with chloroform. Other notches were then filed in the enamel at the point of the interproximal papillae, and the distance between them measured.

To ascertain the *curvative* growth, the same procedure was carried out upon the lingual or cementum side of these teeth. This of course would give the rate of growth in both instances. The file used for

the purpose of notching the teeth was a thin knife-edged tool used by watch-makers for cutting slots in the heads of screws. So much difficulty was experienced in notching the lingual or cementum side of the superior incisors in some of the younger animals employed for this purpose, that it was not carried out in every instance. I think, however, a sufficient number of attempts were successful to establish the fact that the enamel side of the persistent teeth grows much faster than the cementum side; and this, doubtless, is the principal factor in the curvative growth of these teeth.

Donaldson (2) states that he found the average outward (extrusive) growth of the incisor teeth of the rat to be, per week, for the superior, 2.2 mm.; for the inferior, 2.8 mm. Addison and Appleton (1) found the rate of this growth in the mature animal to be the same, that is, 2.2 mm., per week, on an average, for the superior incisors; and 2.8 mm., per week, for the inferior incisors. None of these authors refers to the interstitial or curvative growth.

In our study of the growth of the persistent teeth of the albino rat, we have found that there is a slight variation between the several groups of animals in the growth of these teeth. In the mature animals, ninety to a hundred days old, there is very little difference; while in the young rats, twenty-five to sixty days old, the variation is more noticeable.

The extrusive growth of the inferior incisors in a group of three rats, twenty-five days old on the date of the experiment, was found to be (at the end of seven days) in one animal, 2 mm.; in the two others, 2.5 mm., or an average of 2.3 mm. per week. The curvative growth in the same teeth in the same animals was, for one, 1.8 mm.; in another, 1.5 mm.; while in the third, the notches had been completely worn out by attrition. The curvative growth in this group appeared to be, on an average, about 1.6 mm., a difference between the growth of the enamel (labial) side and the cementum (lingual) side of an average of 0.7 mm.

In another group of two animals aged thirty days (one of which was lost during the experiment by its escape from the cage), the *extrusive* growth of the superior incisor of the remaining animal was 1.5 mm., while the *curvative* growth was 1.0 mm., in this case a difference between the labial and lingual growths of 0.5 mm. The

inferior incisors showed an *extrusive* growth of 3 mm. and a *curvative* growth of 2 mm. per week, a difference of 1 mm.

In still another group of two animals thirty days old, the *extrusive* growth of the inferior incisors in one animal was 2.5 mm. per week. In the second animal of this group, the growth of the teeth, superior and inferior, was so rapid that the notches upon the labial surfaces had completely grown out. The *curvative* growth for the superior incisors in this animal was 1 mm. per week; and for the inferior, 2 mm. The *extrusive* growth for the inferior incisors in this last group was 2.5 mm. per week, and the *curvative* growth was 1 mm., a difference of 1.5 mm. between the labial and lingual sides of these teeth.

IV. SUMMARY OF GENERAL CONCLUSIONS

1. The injection of vital (azo) dyes at various intervals, particularly gentian violet, trypan blue, methylene blue, and naphthamine brilliant blue, leaves definite color lines in the dentine. The distances between these lines show the rate of interstitial growth of this tissue.

2. Alizarine sodium sulphonate is the only *red* vital dye, known to the author, that possesses the power to stain the dentine with definite color lines that can be seen under the microscope.

3. The *interstitial* growth of the dentine of the persistent teeth of the rat, as shown by azo dyes under the microscope, amounts approximately to 0.01 mm. per day.

4. The *extrusive* growth of the superior incisor teeth amounts to about 1.9 mm. per week; of the inferior incisor teeth, to about 2.3 mm. per week.

5. The *curvative* growth of the superior incisor teeth amounts to about 1.0 mm. per week; of the inferior incisor teeth, to about 1.6 mm. per week.

6. The difference between the rates of growth on the labial and lingual sides of the incisor teeth is, on an average, 0.9 mm. more per week on the labial side than on the lingual.

7. Absolute accuracy in measurement is not claimed, but the figures given above are sufficiently precise to form the basis for future study.

V. REFERENCES TO LITERATURE

(1) ADDISON, W. H. F., AND APPLETON, J. L. 1915 The structure and growth of the incisor teeth of the albino rat. *Journal of Morphology*, xxvi, p. 45.

(2) DONALDSON, HENRY H. 1915 The rat, p. 37. Memoirs (No. 6) of Wistar Institute of Anatomy and Biology, Philadelphia, Pa.

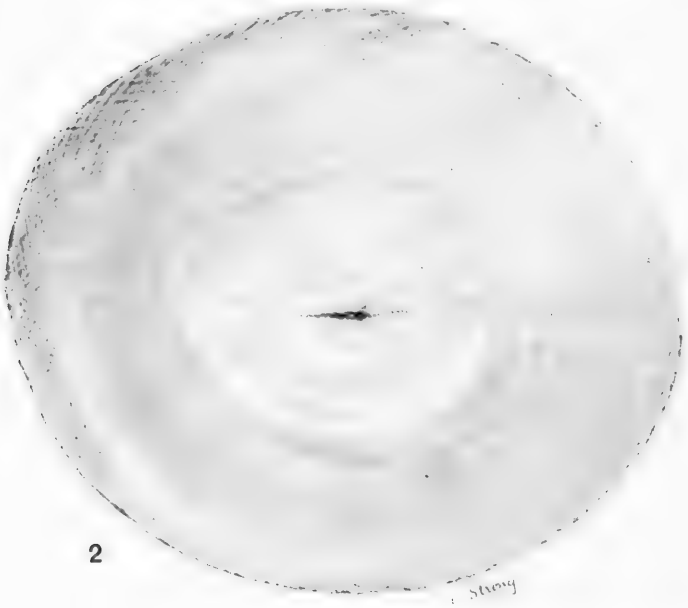
(3) MARSHALL, JOHN S. 1920 An experimental study, with certain vital dyes, of the persistent teeth of the albino rat. *Journal of Dental Research*, ii, p. 1.

PLATE 1

FIG. 1. Drawing of a longitudinal section of the tip of a tusk of an old elephant. Normal size.

FIG. 2. Drawing of a transverse section of a tusk of an old elephant (same as that of *fig. 1*). Sectioned near the distal end of the pulp-chamber. Normal size.

1



2

(Marshall: Interstitial Growth of Rat Incisor Teeth)

PLATE 2

FIG. 3. Drawing of a longitudinal section of a lower incisor of a rat. In situ; × 6.4. Dye: Dianil blue 2R. ("L²;" no. 204.)

FIG. 4. Drawing of a longitudinal section of an upper incisor of a rat. In situ; × 10.7. Dye: Naphthamine brilliant blue. (No. 1,855.)

3

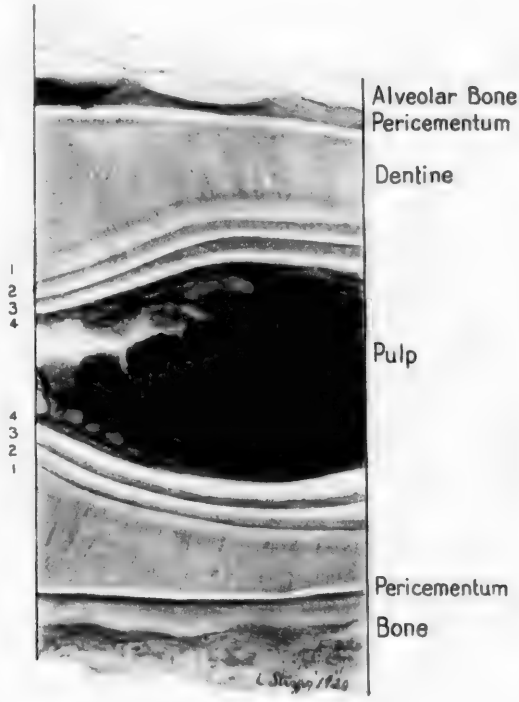
4

(Marshall: Interstitial Growth of Rat Incisor Teeth)

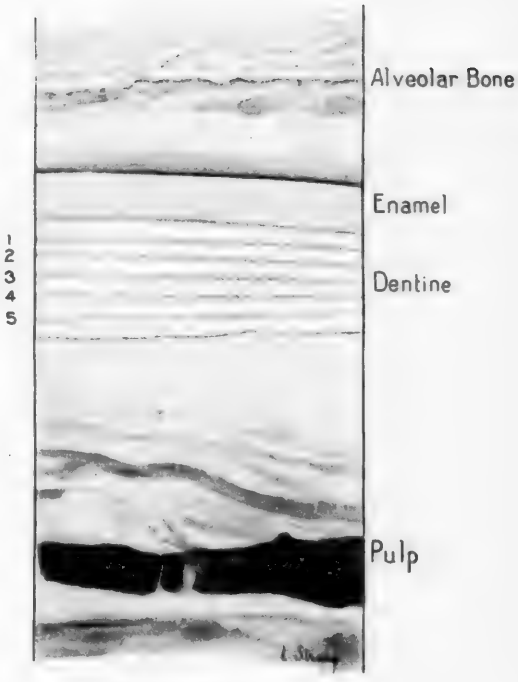
PLATE 3

FIG. 5. Drawing of a part of a transverse section of the root of a persistent lower incisor of a rat showing lines in the dentine; × 50. Dye: Trypan blue.

FIG. 6. Drawing of a part of a longitudinal section of a persistent lower incisor of a rat, showing lines in the dentine; × 50. Dye: Alizarine sodium sulphonate.


Alveolar Bone
Pericementum
Dentine
Pulp
Pericementum
Bone
1 2 3 4
4 3 2 1

5


Alveolar Bone
Enamel
Dentine
Pulp
1 2 3 4 5

6

SOME STUDIES OF CONNECTIVE TISSUES

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I. INTRODUCTION

Of all the elements of the body, connective tissue is the most widely distributed. It has retained its generalized embryonic character and its property of reproduction. It is an emergency tissue and, at need, may be transformed into many of the constituent elements of the body, such as cartilage, bone, blood and blood vessels, and, at times, into elements so closely resembling epithelium as to be called epithelioid tissue. Under certain conditions, and to meet some emergencies, the connective-tissue cell becomes modified so as to assume new functions. One such modification is the bone-building cell, the osteoblast, whose function is to produce bone; another is the osteoclast or bone breaker, whose function is to destroy bone.

II. DEVELOPMENT AND EVOLUTIONARY HISTORY OF CONNECTIVE TISSUE

In order properly to understand the nature of connective tissue, it is necessary to study its development and evolutionary history. Connective tissue arises from the middle germ layer, the mesoderm. In the very early forms of multicellular life this layer does not exist,

[1] The substance of this paper was presented at a meeting of the Harriet Newell Lowell Society for Dental Research, of the Harvard University Dental School, on November 13, 1919.

for the reason that the need for it has not yet arisen. Such a form may be represented by the freshwater hydra. Hydra forms the type of a large group of animals, mostly marine, known as Hydrozoa. The animal consists of a cylindrical body attached by its base to the object upon which it rests. On the free extremity there is a circlet of tentacles at the base of which is situated the mouth, which is constantly receiving from the waving tentacles particles of food in the form of minute animals and plants. Through the mouth these find their way into the primitive digestive cavity, the archenteron, where digestion occurs. The animal consists of two cellular layers: an inner, the digestive one, which also acts as an agent of absorption; and an outer, which comes into contact with the environment and acts as a nervous or sensitive layer (*fig. 1*). The important fact concerning hydra, and the one which is directly involved in this discussion, is the fact that it has only two cellular layers, and needs but two since they come into immediate contact.

Here appears an early expression of organization, very simple and yet effective, inasmuch as it furnishes all the requisite conditions to answer the needs of the animal. Hydra not only occupies a type position in the group of animals to which it belongs but is also, in structure, typical of the early embryonic stage of most animals. In such embryonic forms known as gastrulæ, there are two layers. According to the biogenic law of Haeckel most, if not all, animals pass through this stage during their development; and, according to the same law, all animal life may be arranged in an upward progressive series from protozoa through the metazoa—from the simple diploblastic, or two-layered animals, to the highly organized forms. When the more complicated organisms are considered, the simple two-layered arrangement becomes inadequate. It is no longer possible for the sustaining nutriment to be passed from one cell layer directly to the other. Organization has become established and the necessity has arisen for a means of conveyance of the nutritive ingredients to the various organs and parts of the body. The body no longer consists of two layers in direct contact, but of organs separated by membranes and cavities. This requirement is met by the introduction of a third or middle germ-layer, the mesoderm. In the earthworm such a condition is admirably exemplified. Here organ systems

are introduced and the necessary relations for their interaction have been established. In the gastrula of this animal there is presented what may be interpreted as the inception of the mesoderm. A few cells of the inner layer, the entoderm, become differentiated and, by division, project themselves between it and the outer layer, the ectoderm, until a definite intermediate layer is developed (*fig. 2*).

From the middle germ-layer, or mesoderm, are developed all the connective tissue, bone and cartilage. These are the supporting elements of the body. From this layer are also derived the muscle, blood and blood vessels. The muscle, by its property of contractility, presides over the mechanical movements of the various organs and, by its attachment to the bony skeleton, actuates the movements of the body. The blood and blood vessels become the vehicle by which the food elements are conveyed to the tissues and organs. By this it will be understood that the mesoderm is a sustaining or sustentacular element, created to minister to the higher and more complicated organism in the two capacities of support and sustenance. Of the above derivatives of the mesoderm, the connective tissue, bone and cartilage, are most closely related. Indeed this relation is so intimate that the three have been thrown into a series known as the connective-tissue series, for the reason that both cartilage and bone are developed directly from connective tissue.

III. DISTRIBUTION OF CONNECTIVE TISSUE

It was said, in the beginning, that connective tissue is the most widely distributed element in the body. This is exemplified by the fact that it forms sheaths for the blood vessels, nerves, muscles, bone and cartilage. It fills the intermediate spaces between these elements, and also penetrates the nerves and muscle, and comes into the most intimate contact with their ultimate divisions. This presents for consideration another of the many functional activities of connective tissue. In all these situations it is associated, in the most intimate way, with the blood circulatory system. The particular form of connective tissue here represented is known as areolar, so called because it is filled with spaces between the fibers and fiber bundles. It is also abundantly supplied with capillary blood vessels. The spaces are known as lymph spaces and are filled with lymph,

which is blood plasma after it has been extruded from the capillaries. All the cells of the body come into contact with the areolar connective tissue and therefore with its contained lymph; hence the need for the large amount of connective tissue in nerve and muscle.

What is true of nerve and muscle is true of bone and cartilage. Bone is ensheathed in connective tissue in the form of the periosteum, while the perichondrium bears the same relation to cartilage. If a longitudinal section of compact bone be examined, numerous canals will be seen traversing its substance. These canals will be observed to anastomose so as to form a continuous passage through the bone, from the periosteum to the medullary or marrow cavity. They are known as haversian canals, named from Dr. Havers, who was the first to describe them. They are filled with connective tissue. From the periosteum, capillary blood vessels penetrate into the haversian canals, and are here embedded in connective tissue. There are three vessels within the canal; an artery, a vein and a lymphatic. It will be observed that there is established within the bone a complete system of lymphatic circulation. It may also be remarked that the periosteum holds a very vital relation to this system, since it is the source of the blood supply. The periosteum consists of two layers; an outer layer of dense fibrous tissue with interlacing fibers, in which are situated the larger blood vessels whose branches are distributed to the underlying bone; and an inner layer or, as it may be termed, the inner stratum, which is composed of fibro-elastic, firmly compacted, fibers. In older individuals these fibers are closely attached to the surface of the bone. In the periosteum of developing and growing bone there is a third inner cellular layer, composed of small osteogenic cells known as osteoblasts. This layer is filled with very fine capillary blood vessels. The cells are less numerous in older tissues but always sufficiently numerous to regenerate the bone at need.

IV. RELATION OF PERIOSTEUM TO BONE

The relation of the periosteum to the bone is vital, inasmuch as it preserves the integrity of the bone and, in case of injury, acts as a regenerative agent. This fact has been denied by some authors. One observer has gone so far as to speak of the periosteum as simply

a limiting membrane, and claims that regeneration takes place independently of the periosteal membrane. To say that the periosteal membrane has no osteogenic significance demonstrates, it seems to me, a lack of appreciation of the comprehensive nature of connective tissue. It is certain that the direct agent of bone making is the osteoblast, and it is undeniable that this cell is a modified connective-tissue cell. It is also true that the bone and cartilage are both forms of connective tissue, modified by the deposition of calcium salts within the matrix. Compact bone, so far as the long bones are concerned, is preformed in cartilage. This does not mean that the cartilage is transformed into bone, but rather that it is replaced by newly formed bone. In this process the immediately active bone-forming agent is the periosteum and the resulting product is periosteal bone. The periosteum, being a connective-tissue structure, is the direct source of the osteoblasts that are active in the process of bone construction (*fig. 3*). The bone that is formed to occupy the space originally filled by cartilage is temporary and is removed by the osteoclasts (*fig. 4*). It is only formed as a scaffolding to sustain the structure until the osteoblasts in the periosteum build the bone at the periphery. In the process of bone building, the osteoblasts become imprisoned within the newly formed bone, which they have built up around themselves, and become bone corpuscles (*fig. 5*). Much the same conditions obtain in cartilage, where the connective tissue cells in the perichondrium are directly transformed into cartilage cells. It is also true that when the matrix of hyaline cartilage is broken down in the process of bone development, and the cells are set free, many of them become osteoblasts, which fact is not surprising when it is known that the cartilage cell is a modified connective-tissue cell. These facts demonstrate the unlimited possibilities of connective tissue and the interchangeable nature of connective-tissue cells within the limits of the group to which they belong—the connective-tissue series.

Any cell of areolar connective tissue may, at need, become an osteoblast or a cartilage cell; and *does become* a bone cell when the conditions call for such transformation. Wherever areolar connective tissue comes into contact with bone, and the need for new bone-formation exists, the connective-tissue cells immediately become

osteoblasts and are active in the process of bone building. Just here the universal nature of connective tissue may be considered. When operations requiring the implantation of bone are performed, and the wound is closed, the muscle tissue is wrapped around the introduced bone. A muscle is a mass of contractile tissue imbedded in connective tissue, supplied with abundant capillary circulation. Wherever the connective tissue comes into contact with the bone in the surgical procedure just referred to, the connective-tissue cells at once become modified into osteoblasts as was stated above. *These form a new periosteum*, which presides over the new bone. It must not be overlooked that both blood and blood vessels may be formed from connective tissue. Schäfer has beautifully shown how the connective-tissue cell may form both of these structures (*fig. 6*). This process also takes place when cartilage forms around the point of apposition in mending broken bones, where a cartilaginous ring or callus has formed, only here it is a perichondrium that is formed around the cartilage; and the bone mends just as it was originally developed, by the breaking down of the cartilage matrix and the transformation of the perichondrium into periosteum.

In the development of membrane bone, which is not preformed in cartilage, and which includes most of the bones of the face and skull, the osteoblasts are derived from the connective tissue (*figs. 7* and *8*). It is *always connective tissue* that is involved in the process of bone making and bone can be formed in *no other way*. It is the *periosteum that forms compact bone*, whether in the original membrane or in a new one formed from the epimysial covering of a muscle. Experiments educed to prove the contrary lack some element of careful observation, or interpretation, or both; or they betray inadequate knowledge of the fundamental facts of histology and embryology. Experiments have shown that the detached fragments of periosteal tissue, when placed in favorable environment, will produce bone.

V. CONNECTIVE TISSUE IN RELATION TO THE DEVELOPMENT AND STRUCTURE OF THE TOOTH

The development and structure of the tooth presents another example of the versatile character of connective tissue. The entire

tooth is developed from this tissue. In its formation two kinds of cells, both modified from the connective-tissue cell, are called into action: the odontoblastic cell forming the dentine and the osteoblastic cell forming the cementum, the former being produced from the connective tissue in the dentinal papilla, the latter from the same tissue in the alveolo-dental periosteum. The enamel is a secondary epithelial structure deposited upon the already formed dentine for the purpose of protection. The enamel is of ectodermal origin, while the entire tooth arises from the connective tissue, which is mesodermal.

One might speak of the tooth as consisting of dentine and pulp, since the enamel and cementum are accessory additions. Cementum is the product of the activity of the osteoblastic cells, sometimes, although needlessly, called cementoblasts. During development the sacculus wall, or outer membrane of the dental follicle, is projected from the horns of the enamel organ and covers the outside of the elongating tooth root. The cells of this connective-tissue membrane become osteoblasts and deposit on the forming root a layer of bone. This is the cementum. The membrane becomes the alveolo-dental periosteum.

The cementum is thickest at the apex of the root, where it shows successive incremental layers between which are situated minute spaces or lacunae, in which are contained cells or corpuscles. These structures are analogous, if not identical, with like structures in the compact bone. From the lacunae project minute canals (canaliculae) comparable with the same elements in bone.

Hopewell-Smith denies the existence of lacunae in cementum, claiming that they occur only in cementum which has undergone hyperplasia. It is somewhat difficult to understand how this can be, since the development is the same in both instances. Both are the products of the activity of the osteoblastic cells in the alveolo-dental periosteum; and in the process, in both cases, the osteoblasts become imprisoned within the bone which they have formed. The absence of lacunae in one and not in the other would be hard to reconcile. Moreover, the statement made by Dr. Hopewell-Smith contradicts the published opinions of all the histologists who have written on the subject. After years of study of these structures I am much inclined to agree with the majority. *Figure 11* shows not

only lacunae in the cementum but also seems to agree with the statement made and illustrated by Kölliker: that the dentinal fibril reaches the lacuna through the much-elongated dentinal tubule.

The feature in this connection which is of particular interest relates to the protean character of the connective-tissue cell. Here it is modified into an osteoblast, while on the surface of the dentinal papilla it becomes an odontoblast. The former produces the bony structure of the cementum; the latter forms the dentine and at the same time furnishes the dentinal fibril, which fills the dentinal tubule and carries living protoplasm throughout the tooth substance. In both cases the cells persist through adult life, the osteoblast in the alveolo-dental periosteum and the odontoblast on the peripheral surface of the pulp. Not only do they exist as integral elements of their respective structures but, when stimulated, they also revive their primitive functional activities and form their characteristic products. Under these conditions the osteoblast in the alveolo-dental periosteum produces additional cementum known variously as exostosis and hyperplasia. The odontoblasts under like conditions form pulp concretions and secondary dentine. There is some question as to whether these secondary structures within the pulp are formed by the odontoblasts or by the connective-tissue cells; but, in either case, the result is the same, as they are both connective-tissue cells.

The alveolo-dental periosteum presents some features that illustrate the versatile character of connective tissue. The fibers of this membrane are divided into groups, in one of which the fibers are so joined as to form a ligament composed of white non-elastic elements having great strength. They are projected into the cementum in the form of the so-called fibers of Sharpey, where they become calcified and so act as an anchorage to the tooth. They pass directly across from the alveolar bone to the tooth, thus suspending the latter within the alveolar socket, giving the necessary play to relieve the shock incident to the force of mastication. Other groups of delicate fibrils act as support to the vascular and nervous elements which are abundantly supplied to this important adjunct to the tooth. In *figs. 9* and *10*, this is illustrated. In *fig. 9*, at *a*, the strong fibers are shown and, at *b*, the finer or more delicate fibrils are presented. *Figure 10* shows the structure in situ.

Thus far, in dealing with the alveolo-dental periosteum, only the mechanical aspect has been considered but the vital function is quite as important, if not more so. The membrane is richly vascular, receiving its blood supply from the same source as does the pulp. It is said by some authors that capillaries are not numerous, but that the circulation is "arterial and venous." This proposition is hardly clear in the light of our present knowledge of lymphatic circulation. It is difficult to comprehend how the tissue could be nourished if the blood remained constantly within the vessels as, I understand, the statement suggests. This situation bears some resemblance to the old controversy over the presence or absence of lymphatic vessels in the dental pulp. A more complete understanding of lymphatic circulation, and the part played in it by the connective tissue, would have suggested, long ago, a solution of this difficult problem and demonstrated that the presence of lymphatic vessels in the dental pulp is not *necessarily* essential. The connective tissue itself is the active factor in this vital activity; and it is quite possible that the tissue is drained into the lymph vessels outside, in the alveolo-dental periosteum. Several observers claim to have found lymphatic vessels in the pulp but their results are not convincing; and, until further research work is done in this difficult field, the subject must be held in abeyance. It is well known that conditions analogous to those suggested above exist in the voluntary muscle, which contains no lymphatic vessels but is drained directly into the enveloping connective tissue.

The histologic structure of the alveolar bone, and its relation to the alveolo-dental periosteum, is a subject too little understood or considered. The bone belongs to the cancellous variety and is full of spaces, like all bone of its kind. Into these spaces the connective tissue of the alveolo-dental periosteum projects, filling them with this tissue. Obviously any infectious condition in the one may be, and probably is, communicated to the other. This may explain the fact that, in cases of pyorrhea alveolaris, the alveolar bone is destroyed; and also may prove to be the reason for the persistence of the disease, after all possible instrumentation in the alveolar process has proved unavailing (*fig. 12*).

Associated with the tooth is another cell of connective-tissue origin, the osteoclast. This cell has for its function the destruction of bony tissue. It is a so-called giant cell and is multinucleated. Kölliker found in the largest of these cells as many as fifty nuclei. They are responsible for the so-called resorption of the deciduous tooth and also for the same process with the alveolar bone after extraction. They are probably developed from osteoblasts by repeated nuclear division.

VI. GENERAL CONCLUSIONS

The facts here presented point clearly to the protean character of connective tissue, and also illustrate how the purpose for which the middle germ-layer was originally developed has been achieved in the higher organisms. These facts also demonstrate that the supporting and sustaining components of the body are included in a definite group of structures, in which connective tissue is the fundamental element and the other members of the group are modifications of this basal constituent.

PLATE 1

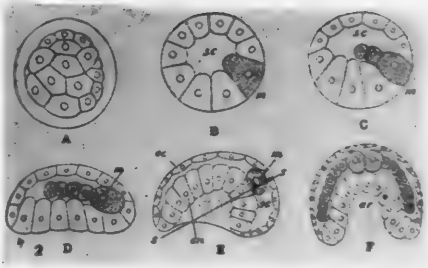
FIG. 1. *Hydra fusca*, showing two layers. After Marshall and Hurst.

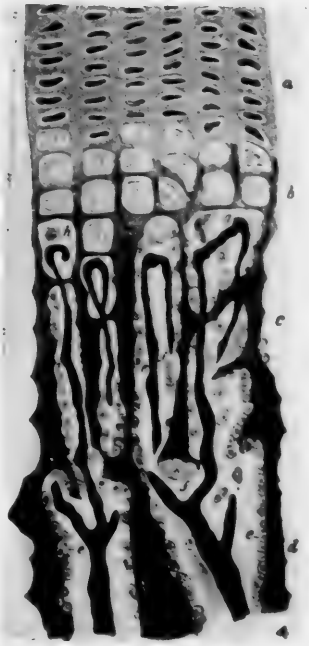
FIG. 2. Gastrula of the earthworm. *A*.—Morula. *B*.—Section of morula, showing first mesodermic cell. *C*.—Mesodermic cells dividing. *D* and *E*.—Gastrula forming. *F*.—Mesoderm formed.

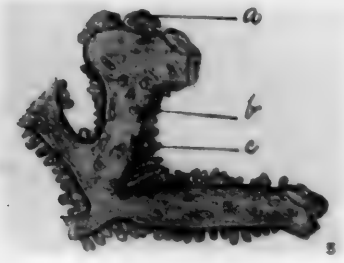
FIG. 3. Longitudinal section through part of a phalanx of a human embryo at six months. After Kölliker. *a*, calcified cartilage; *b*, periosteal bone; *c*, embryonic marrow; *d*, periosteum.

FIG. 4. Longitudinal section of the developing femur of a rabbit. After Klein. *a*, rows of flattened cartilage cells; *b*, degenerating cartilage cells; *c* and *d*, formed bone covered with osteoblasts; *f*, osteoclast beginning to tear down the bony spiculae. Osteoblasts are seen everywhere on the spiculae.

FIG. 5. Bony trabecula from the lower jaw of a calf embryo, showing osteoclasts, with Howship's foveolae at *a*. After Kölliker. *c*, osteoblasts; *b*, bone corpuscles and lacunae.







(Bates: Studies of Connective Tissues)

PLATE 2

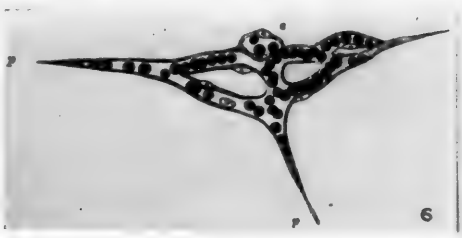
FIG. 6. Formation of blood and blood vessels from connective-tissue cells. After Schäfer.

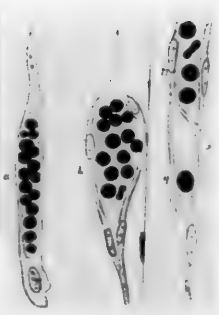
FIG. 7. Section of ossifying maxillary bone of new-born rat. After von Korff. *b*, bony substance with osteoblasts and fibers; *b'*, growing border of bone; *c*, embryonic connective tissue, showing its fibers continuous with the osteogenic fibers of the growing border; *o*, osteoblasts.

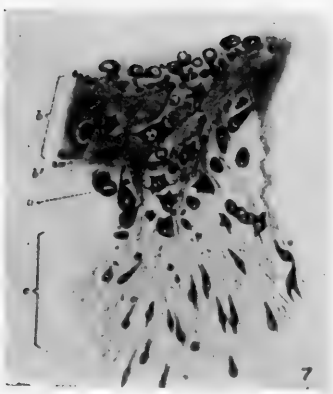
FIG. 8. Development of membrane bone. After Schäfer. *a*, granules of calcareous deposit between fibers; *b*, granules have become blended; *of*, osteogenic fibers with osteoblasts between them; *ost*, osteoblasts imbedded in the newly formed bone; *sp*, bony spicules with osteoblasts imbedded in them, producing lacunae.

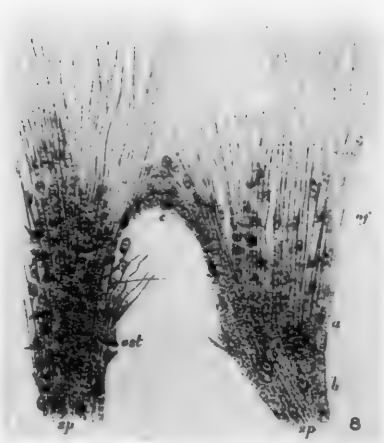
FIG. 9. Strong fibers passing from cementum (*a*) to alveolus (*b*). After Black. In the lower figures, the fibers are broken up into fine strands.

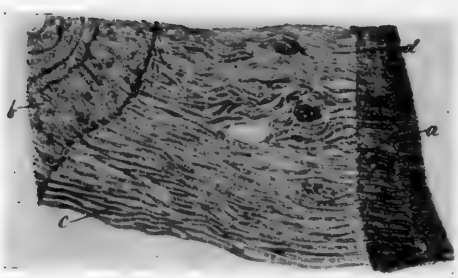
FIG. 10. Section of tooth root in situ. After Black. *a*, dentine; *b*, cementum; *c*, pulp; *d*, alveolar bone; *e*, alveolo-dental periosteum, showing direction of fibers; *g*, hypertrophy of cementum.

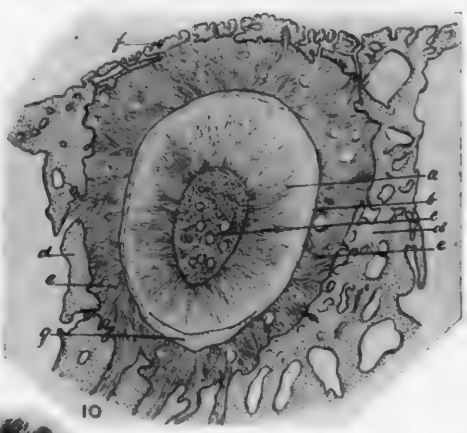


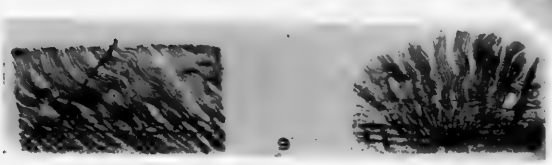












(Bates: Studies of Connective Tissues)

PLATE 3

FIG. 11. Photomicrograph of a section of a tooth root, showing the arborescence of the dentinal tubules. Some of the tubules extend into the lacunae of the cementum. The presence of lacunae in the cementum is also demonstrated.

FIG. 12. Photomicrograph showing the relation of the alveolo-dental periosteum to the alveolar bone. *a*, dentine of the tooth; *b*, alveolo-dental periosteum; *c*, alveolar bone; *d*, connective tissue projected into the cancellous bone from the alveolo-dental periosteum.

(Bates: Studies of Connective Tissues)

SOME AMERICAN PLANTS CONSIDERED AS SOURCES OF VITAMINES, AND AS PARTS OF A DIET FAVORABLE TO THE PRESERVATION OF THE TEETH[1]

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I. INTRODUCTION

It seems evident, from data compiled by Pickerill, that savage races have very good teeth, whereas about 95 percent of the number of civilized persons have dental caries; and also that this condition is not due to heredity, since the change in the teeth follows the process of civilization, no matter how rapidly this process is effected. That diet has something to do with tooth preservation seems probable, since a pyorrhea-like affection is characteristic of scurvy and the teeth become imperfect as a result of rickets. Dr. Edwin F. Robb,[2] working in my laboratory, has shown that in scurvy the calcium stores of the body are depleted, and that much calcium passes out in the urine. The bones dissolve by enlargement of the marrow cavities. I have found osteodentine in the pulp cavity in scurvy.[2]

[1] This paper was received for publication on August 29, 1920, and is one of many available papers that we have not been able to publish promptly. This paper was referred to, in the last preceding issue, as due for publication in this number (McClendon and collaborators: JOURNAL OF DENTAL RESEARCH, 1921, iii, p. 40).—ED.

[2] Robb, McClendon, and collaborators: JOURNAL OF DENTAL RESEARCH, 1921, iii, p. 39.

Until we know more about the nature and causes of the "deficiency" diseases, we cannot determine how prevalent they are. Many ill defined illnesses may be incipient cases of "deficiency" diseases. With the possible exception of pellagra (and related diseases, such as acrodynia), which seems to depend on protein deficiency, all the "deficiency" diseases appear to be due to lack of some chemical substance of unknown composition, called vitamine for lack of a better name. Durand found dental caries in a larger percentage of children that had been fed in infancy on sweetened condensed milk, than in children that had been breast fed. Condensed milk has a low vitamine content, due to partial destruction of vitamines by heat and to dilution of vitamines with a vitamine-free substance, sugar.

It seems very probable that, if we knew all the foods eaten by savage races and ate them in the amounts eaten by savages, the incidence of dental caries would be greatly decreased. This opinion specially includes the diet of the infant during the period when the teeth are developing. Since savages had practically no "artificial" infant food except the banana, which is said not to be indigenous to America and certainly was not to the greater part of North America, American Indians must have nursed their infants for a long period. It is said that the Fijians nursed their infants three years and gave them bananas and other things in addition. It seems necessary to conclude that the infants of American Indians could not have survived if the period of nursing had not been as long as that.

Ethno-botanists list a number of plants that were and are eaten by children of American Indians. While the papoose was not allowed to crawl around, it must have been dependent on what was given to it. I have seen mothers chew food until it was soft and then give it to infants to eat. This method of feeding was probably common among savages. The fact that some infants, that have been breast fed for three years, are undernourished, does not indicate the presence of deleterious substance in the milk, but rather an insufficiency of some substance that could be supplied by the suitable *addition* of other food, without the necessity for weaning.

I had occasion to make observations of the diet and teeth of Mexicans, during the years from 1888 to 1903, in various parts of Mexico

and on the Mexican border. The Mexican has good teeth. The conclusion from these casual observations of the teeth of the Mexican—that they are good teeth—is corroborated by the records of the U. S. Army Draft Boards, in the contrast of the poor teeth of the general draftees with the excellent teeth of the draftees along the Mexican border that included men of Mexican descent. As to the food eaten by Mexicans: my observations, coming long after the Spanish settlers had introduced sugar-cane, peaches, oranges and other plants, do not relate to the primitive diet, but they indicate that certain accounts that have been published about that diet are misleading. Hopewell-Smith states that "the Indians of Mexico seldom employ condiments for flavoring food, which is generally simple in character and taken cold." On the contrary, the further I travelled from railroad communication, the better flavored I found the diet of the Mexicans. When we bear in mind that yuccas and nolinas, such as Dasylerion texanum, too woody for a horse or cow to eat, are used by Mexicans for making beverages, the impression will be gained that the Mexican uses everything possible for food and drink. We now know that yeast is a rich source of water-soluble vitamine. The Mexicans imbibed cultures of wild yeast in the fermented pulque, sotol, and other drinks. It was only after the advent of the white man that these nutritious drinks were distilled to form the mescal, tequila, and sotol aquardiente, that have largely replaced the former.

Besides eating food from nearly every phylum of the animal and plant kingdoms, the Indians imbibed a large number of drinks made by extracting plants with hot or cold water and hence containing water-soluble vitamines. Since the North American Indians seem to be the best known in this regard, a (partial) list of the plants they used is given below, followed by a list of tropical American plants that have found their way into the United States and may have done so occasionally in pre-Columbian days. Some of these plants were cultivated by North American Indians, especially corn, beans, pumpkins and squashes. Since it is immaterial from a nutritional standpoint whether vitamines are taken as food or medicine, these two groups of plants are not separated in the appended lists (1 and 2).

II. LISTS OF AMERICAN PLANTS

List No. 1. Some North American plants that were used as food, or taken internally as medicine, by North American Indians; including some materials that were "chewed" and their vitamines swallowed

Abronia fragrans (sand puff)
Acanthocheton wrightii (herb, ancient Hopi food)
Acer negundo, saccharum, saccharinum and rubrum [maple (sugar)]
Achillea millefolium (yarrow)
Acornus calamus (sweet flag)
Actinella odorata (herb)
Adenostegia (yumayut)
Agaricus campester and arvensis (mushroom)
Agastache anetheodora (anise)
Agave americana, parryi, palmeri, wislizeni, and deserti (maguey, pulque plant)
Agrostis perennaus (grass)
Alectoria jubata and fremontii (lichen)
Allionia nyctaginea (four-o-clock)
Allium bisceptrum, acuminatum, mutabile, recurvatum, and cernuum (wild onion)
Amanita rubescens (mushroom)
Amaranthus blitoides, torreyi and retroflexus (tumbleweed)
Ambrosia elatior and artemisiaefolia (ragweed)
Amelanchia alnifolia (June berry, service berry)
Ammobroma sonorae (sand food)
Amoreuxia wrightii, and palmatifida (sayas, indian parsnip)
Amorpha canensens (shoestring)
Amsinckia tessellata (herb)
Andropogon furcatus (red hay)
Anemone canadensis and cylindrica (wind flower)
Angelica (angelica)
Anhalonium engelmanni, fissuratum and lewini (cactus, peyote)
Aphlopappus (herb)
Aphyllon fasciculatum (cancer root)
Apios apios, and tuberosa (ground nut)
Aquillegia coerulea, and canadensis (wild columbine)
Arctostaphylos manzanita, patula, glauca and tomentosa (manzanita)
Arisaema triphyllum (indian turnip)
Armillaria mellea (honey mushroom)

Artemisia biennis, discolor, trifida, dracunculiodes, tridentata, frigida, gnaphalodes, forwoodii, filifolia, wrightii, and heterophylla (wild sage, fuzzy weed, wormwood)
Asclepias tuberosa, exaltata, syriaca, verticillata, erosa, speciosa, eriocarpa, galioides, and involucrata (butterfly weed, milkweed)
Asclepiodora decumbens (milkweed)
Asimina triloba (North American pawpaw)
Aster hesperius (wild aster)
Astragalus caroliniana, pictus filifolius and diphysus (rattle pod, milk vetch)
Atriplex canescens, confertifolia, argenteum and powellii (arache)
Avena fatua (wild oats)
Bahia woodhouseii [(purgative) thistle]
Balsamorrhiza sagittata (arrow root)
Barbarea barbarea, stricta and præcox (scurvy grass)
Bechmannia erucaeformis (slew grass)
Berberis canadensis, repens, aquilifolium and trifoliata (barberry, Oregon grape)
Betula lenta, and fontinalis (birch)
Bidens bigelovii (bur marigold)
Bigelovia douglasii and parishii (rabbit bush)
Blennosperma californicum (weed)
Bloomeria aurea (lilly)
Boletus versipellis and edulis (mushroom, cepe)
Bovista plumbea (puff ball)
Brickellia grandiflora and wrightii (herb)
Brodiaea congesta, capitata and volubilis (purple-flowered grass-nut, climbing grass-nut, wild hyacinth)
Bromus breviaristatus and maximus (brome grass)
Cactus viviparus, heyderi and goodrichii (cactus)
Calandrinia cawlescens (red maids)
Calla palustris (water arum)
Calliproa lutea (yellow-blossom grass-nut)
Callirrhoe involucrata, digitata and pedata (mallow)
Calochortus nutallii, macrocarpus and aureus (mariposa lilly, sego)
Calvatia cyathiformis, and gigantea (puff ball)
Camassia esculenta (camos)
Cantharellus cibarius (mushroom)
Carduus ochrocentris (thistle)
Carex utriculata (sedge)

Carcum gairdnerii, kelloggii and oregonum (yamp, wild anise)
Castanea dentalla and pumila (chestnut)
Castanopsis chrysophilla (Oregon chinquapin)
Castilleia parviflora (painted cup)
Caulophyllium thalictroides (blue cohosh)
Ceanothus americanus (red root, New Jersey tea)
Celtis occidentalis and reticulata (hackberry)
Cercocarpus montanus (mountain mahogany)
Cereus giganteus, thurberi, stramineus and triangularis (giant cactus)
Chamaesaracha coronopus (benedictine berry)
Chamaesyce serphylliflora (milkweed)
Chenopodium capitatum, leptophyllum, rubrum, cornutum, fremontii, californicum and album (lambsquarters, pigweed)
Chlorogalum pomeridianum, parviflorum and divaricatum (little soap-root, wild potato)
Chrysopsis villosus (golden aster)
Cinna arundinacia (wood reed-grass)
Citrullus citrullus (watermelon)
Clavaria flava and fusiformis (coral mushroom)
Claytonia caroliniana, virginica, megarrhiza, and perfoliata (wild lettuce, spring beauty)
Clematis lingusticifolia (virgin's bower)
Cleome integrifolia (stinking clover)
Clitopilus aboritivus (mushroom)
Cnicus drummondi, undulatus and edulis (thistle)
Collybia velutipes and radicata (mushroom)
Coprinus comatus, micaceus and a tramentarius (shaggy-mane mushroom, glistening coprinus, inky coprinus)
Coreopsis cardaminefolia (coreopsis)
Coriandrum sativum (coriander)
Corylus americana and californica (hazelnut)
Cowania mexicana (cliff rose)
Crataegus chrysocarpa (red haw)
Cripis glauca (hawk's beard)
Croton texensis, corymbulosus (croton, chapparal tea)
Cucurbita ficifolia, and foetidissima (gourd)
Cyclobothra (beaver-tail grass-nut)
Cycloloma atriphicifolium (winged pigweed)
Cymoterus longipes, montanus, globosus, glomeratus, fendleri and purpureus (chimaja)

Cyperus esculentus and rotundus (chufa, nut grass)
Dalea lanata (herb)
Dasylerion texanum (sotol)
Dasystephana puberula (gentian)
Datura meteloides and quercifolia (jimson weed)
Deschampsia caespitosa (hair grass)
Dichoria brandegii (katokia)
Diospyros virginiana and texana (persimmon)
Dithyraea wislizeni (spectacle pod)
Dracocephalum parviflorum (mint)
Echeveris lanceolata (rock lettuce)
Echinocactus visnaga and wislizeni (cactus)
Echinocystis macrocarpa (chillicothe)
Elaegnus argentea (silver berry)
Elymus canadensis and condensatus (wild rye)
Ephedra nevadensis and antisyphilitica (teamster's tea, joint fir)
Epilobium coloratum (willow herb, fireweed)
Equisetum laevigatum (horsetail)
Eriocoma membranacea and cuspidata (grass)
Eriodictyon tomentosum and parryi (yerba santa)
Eriogonum ovalifolium, corymbosum, alatum, fasciculatum and jamesii (medicine root)
Eryngium aquaticum (button snakeroot)
Erythraea venusta (canchalagua)
Erythronium albidum and mesochoreum (spring lilly)
Escholtzia californica (California poppy)
Euonymus atropurpurea (burning bush)
Euphorbia serphyllifolia and polycarpa (spurge)
Euretia lanata (white sage)
Falcata comosa (ground bean, hog peanut)
Ferula multifida (totuv)
Festuca ovina (sheep's fescue-grass)
Fistulina hepatica (beef-tongue mushroom)
Fragaria vesca, americana and virginiana (strawberry)
Fritillaria atropurpurea and pudica (lilly)
Gaertneria acanthicarpa (ragweed)
Galium (bedstraw)
Gaultheria procumbens (wintergreen)
Gaura parviflora (herb)
Gaylusacia (huckleberry)

Geranium fremontii (cranesbill)
Geum macrophyllum (herb)
Gilia aggregata and staminea (standing cypress)
Glyceria distans (manna grass)
Glycine apios (indian potato)
Glycyrrhiza lepidota (licorice)
Grindelia squarrosa (sticky head)
Grossularia missouriensis (gooseberry)
Gutierrezia euthamniae, filifolia and longifolia (broom weed, snake weed)
Gymnolemia multiflora (herb)
Hedeoma hispida and drummundii (pennyroyal)
Hedysarum mackenzii (legume)
Helianthus annuus, tuberosus, doronicoides, and giganteus (sunflower, Jerusalem artichoke)
Heracleum lanatum (cow parsnip)
Hesperocallis undulata (desert day-lilly)
Hesperoscordium lacteum (white-flowered grass nut)
Heteromeles arbutifolia (christmas-berry)
Heuchera pubescens (alum root, rock geranium)
Hicoria ovata (hicory nut)
Houttuynia californica (yerba mansa)
Humulus americana (hops)
Hydnum coralloides, laciniatum and erinaceus (coral mushroom)
Hymenopappus filifolius (thistle)
Hymenoxys floribunda (Colorado rubber-plant)
Ilex vomitoria (cassine)
Ionoxalis violacea (sheep sorrel)
Iva axillaris (marsh elder)
Juglans nigra and cinera (black walnut, butternut)
Juniperus californica, virginiana, occidentalis, pachyphloea, mexicana and monosperma (cedar, juniper)
Kalmia glauca (pale laurel)
Kryntzkia sericea (herb)
Kunzia tridentata (shrub)
Laciniaria scariosa and punctata (blazing star)
Lactarius deliciosus (orange milk-mushroom)
Lactuca pulchella (blue-lettuce)
Lathyrus (pea)
Layia glandulosa (tidy-tips)
Ledum palustre and groenlandicum (marsh-tea, labrador-tea)

Lepargyrea argentea (buffalo-berry)
Lepidium nitidum (peppergrass)
Lepiota procera (parasol mushroom)
Leucelene ericoides (thistle)
Lewisia rediviva and brachycalyx (bitter-root)
Lilium umbellatum (lilly)
Lindera benzoin (spice-bush)
Linum lewisii, rigidum and puberulum (flax)
Lithospermum pilosum, multiflorum and canencens (puccoon)
Lonicera conjugialis (Oregon cranberry)
Lophanthus urticifolius (mint)
Lophophora williamsii (cactus)
Lotus strigosus (tovinal)
Lupinus littoralis (lupine)
Lycium pallidum (tomatillo)
Lycoperdon gemmatum, piriforme, and solidum (puff ball, indian bread)
Lygodesmia juncea and grandiflora (skeleton weed)
Madaria (tar weed)
Madia glomerata (tar weed)
Malacothrix californica (makiyal)
Malus ioensis (crabapple)
Malvastrum coccineum (red false-mallow)
Mammilaria (ball cactus)
Marasmius oreades, scorodonius and rotula (fairy-ring mushroom, garlic mushroom, little-wheel mushroom)
Martynea louisiana (unicorn plant)
Medeola virginiana (indian cucumber-root)
Melica (melic grass)
Mentha canadensis (mint)
Mentzelia albicaulis (herb, stick leaf)
Mesembryanthemum aequilaterale (fig marigold)
Micromeria douglassii (yerba buena)
Microseris nutans (succulent root)
Mimulus luteus (mask flower)
Mirabilis californica (herb)
Mitchella repeus (partridge berry)
Mitella trifida (bishop's cap)
Monarda fistulosa and citriodora (horsemint)
Monardella lanceolata (indian tea)
Montia perfoliata (indian lettuce)

Morchella esculenta, deliciosa, conica and semi-libera (morel)
Muscadinia munsoniana and rotindifolia (bullace grape, muscadine, scuppernong)
Myrica asplenifolia (sweet fern)
Nelumbo lutea (yellow lotus, water chinquapin)
Nymphaea polysepala and advena (yellow pond-lilly)
Oenothera biennis and caespitosa (evening primrose)
Opuntia rutila, polyacantha, humifusa, tuna, ficus-indica, engelmannii, vulgaris, arborescens, camanchica and whipplei (prickly-pear, cane cactus)
Orobanche tuberosa (cancer root)
Orogenia linearifolia (wild parsnip)
Orontium aquaticum (golden club)
Oxycoccus macrocarpus(cranberry)
Padus nana and melanocarpa (chokecherry)
Panicularia fluitans (sugar grass)
Panicum obtusum and capillare (old-witch grass)
Parmelia saxicola (lichen)
Parosela aurea and lasianthera (bitter medicine)
Passiflora incarnata (maypop)
Pectis angustifolia and papposa [thistle family]
Pellaea omithopus (tea fern)
Peltandra virginica and sagittaefolia (arrow arum)
Pentstemum grandiflorus (fox glove)
Peritoma serrulatum (rocky-mountain bee-plant)
Petalostemum purpureum (purple clover)
Peucidanum graveolens, canbyi, eurycarpum, farinosum, geyeri, and ambiguum (biscuit root)
Phacelia ramosissima (sikimona)
Philibertia beterophylla (milkweed)
Pholiota praecox and caperata (mushroom)
Phorandendron juniperium (mistletoe)
Photinea arbutifolia (California holly)
Phragmites phragmites (cane grass)
Physalis neomexicana, fendlerii, longifolia, heterophylla and lanceolata (ground cherry)
Physaria newberryi (hohoyana)
Pinus edulis, monophylla, cembroides, ponderosa, lambertiana, sabiniana and murrayana (pine nut, piñon, sugar pine)
Pleurotus ulmarius, ostreatus (mushroom, oyster mushroom)

Poa californica (meadow grass)
Podophyllum peltatum (may apple)
Polygonum ramosissimum, douglasii and lapathifolium (smartweed)
Polyporus applanatus and frondosus (tree fungus)
Polystictus versicolor and perennis (bracket fungus, mushroom)
Populus sargentii and tremuloides (cottonwood, aspen)
Portulaca retusa and oleracea (purslane)
Promus virens (grass)
Prosopis juliflora and pubescens (mesquite, screw bean)
Prunus demissa, americana, besseyi, nigra, angustifolia, subcorticata, ilicifolia and hortulana (plum)
Pseudotseuga douglassii (douglass spruce)
Psoralea esculenta, tenuiflora, hypogaea, californica, castorea, canescens, orbicularis and subacaulis (tipsin, indian potato)
Pteris aquilina (brake)
Quamasia quamash (camos)
Quamoclidion multiflorum (wild four-o-clock)
Quercus undulata, macrocarpa, rubra, garryana, lobata, virginiana, pungens, oblongifolia, engelmannii, michauxii, prinoides, agrifolia, gambelii, douglasii, californica, chrysolepsis, wizlizeni, dumosa (oak)
Ramona stachyoides and polystachya (black sage and white sage)
Ramunculus aquatilis, cymbalaria and californicus (crow foot)
Ratibida columaris (cone flower)
Reverchonia arenaria (patanwuba)
Rhamnus purschiana (cascara sagrada)
Rhus trilobata, aromatica, glabra, toxicodendron, hirta, copallina, integrifolia and ovata (sumac and poison ivy)
Ribes aureum, divaricatum, lacustre, leptanthum, oxyacanthoides, americanum, cereum and inebrians (currant)
Rosa californica, fedlerii, pratincola and nutcana (wild rose)
Rubus leucodermis, nutkanus, occidentalis, stragosus parviflorus and vitifolius (raspberry, blackberry)
Rumex hymenosepalus, geyerii, salicifolius and mexicanus (dock)
Russula virescens (mushroom)
Sabal palmetto (palmetto)
Saccharomyces (yeast)
Sagittaria latifolia and arifolia (arrowleaf)
Salicornia herbacea (brittlewort)
Salvia polystachya, columbariae, carduacea, tiliaefolia, ballatæflora (sage, chia)

Sambucus racemosa, glauca and canadensis (elderberry)
Sanicula tuberosa (turkey pea, snakeroot)
Sassafras officinale (sassafras)
Sidalcea malvaeflora (wild hollyhock)
Scirpus lacustris and validus (bulrush)
Scutellaria (skullcap)
Senecio (squaw weed)
Sericotheca dumosa (a small, round fruit)
Sheperdia argentea and canadensis (bullberry, soapberry)
Silene acaulis, multicaulis and scouleri (catchfly)
Silphium perfoliatum, laciniatum (cup plant, pilot weed)
Sisymbrium canescens and incisum (hedge mustard)
Sisyrinchium bellum (blue-eyed grass)
Sium cicutaefolium (herb)
Smilax herbacea, pseudo-china, bona nox, glauca, rotundifolia and auriculatum (Jacob's ladder)
Solanum jamesii, elaegnifolium, fendlerii, triflorum, douglasii and rostratum (nettle, buffalo bur, native potato, nightshade)
Solidago canadensis, nemoralis, spectabilis, missouriensis and odora (goldenrod)
Sonchus asper (saw thistle)
Sophia (tansy mustard)
Sophora secundifolia (frijolillo)
Sparassis herbstii (mushroom)
Sparganium eurycarpum (bur reed)
Spathyema foetida (polecat weed)
Sphaeralcea angustifolia, and incana (mallow)
Spiraea caespitosa (spiraea)
Sporobolus cryptandrus (rush grass)
Stachys palustris (wound wort)
Stanleya albescens (wild mustard)
Stanleyella wrightii (wild mustard)
Suaeda depressa (sea blite)
Talinum aurantiacum (herb)
Thelesperma gracile and trifidum (indian tea)
Townsendia arizonica (herb)
Tricholoma equestre (mushroom)
Trifolium ciliolatum, gracilentum, microsephalum, tridentatum, obtusiflorum and other species (clover)
Triglochin maritimum (arrow-grass)

Tripterocalyx wootonii [four-o-clock family]
Trisetum subspicatum (grass)
Triticum vulvage (plant)
Troximon aurantiacum (indian water-cress)
Typha latifolia (cattail)
Ulmus fulva (slippery elm)
Ustilago maydis or zeae (corn smut)
Vaccinium caespitosum, membranaceum, scoparium and many other species (blue-berry, bilberry)
Vagnera amplexicaulis (false solomon's-seal)
Valeriana adulis (kooyah)
Verbena hastata (verbena)
Viburnum lentago (black-haw)
Viola cucullata and pedunculata (violet)
Vitis arizonica, labrusca, rupestris, girdiana, monticola, cordifolai, bicolor, candicans and other species (wild grape, fox grape, sugar grape, mustang grape)
Washingtonia longistaylis (sweet cicely)
Woodwardia radicans (brake fern)
Wyethia amplexicaulis (succulent root)
Xanthium commune (cocklebur)
Xanthoxalis stricta (sourgrass)
Ximenesia exauriculata (crownbeard)
Yucca baccata, macrocarpa, treculeans, schottii, whipplei, mohavensis and glauca (yucca, soapweed, spanish bayonet)
Zizania aquatica (wild rice)
Zygadenus nuttalli (lilly)

List No. 2. Some tropical American plants that were cultivated by North American Indians, or are suitable articles of commerce from the standpoint of content of vitamines

Achras sapota (chewing-gum tree, sapodilla)
Ananas ananas (pineapple)
Anona cherimolia, reticulata, muricata, and squamosa (cherimoya, custard apple, sour sop and sweet sop)
Arachis hypognea (peanut)
Batatas batatas (sweet potato)
Bertholletia excelsa (brazil nut)
Brosimum alicastrum (bread nut)

Capsicum annuum (red pepper)
Casimiroa edulis (white sapota)
Cerica papaya (papaya)
Chenopodium quinoii (quinoa)
Chrysophyllum cainito (star apple)
Coccoloba uvifera (sea grape)
Cocus nucifera (coconut)
Coumarouna odorata (tonka bean)
Cucumis angurica (gherkin)
Cucurbita pepo and maxima (pumpkin, squash)
Cryptocarya moschata (Brazilian nutmeg)
Erythroxylon coca (coca plant)
Eugenia pimenta (Jamaica pepper)
Ilex paraguayensis (mote)
Lecythis zambucayo and amoxonum (monkey pot, paradise nut)
Lucuma mammosa (lucuma)
Lycopersicon lycopersicum (tomato)
Mammea americana (mammee apple)
Manihot manihot, and palmata aipi (cassava)
Maranta arundinacea (arrowroot)
Paullinia cupana (guarana)
Pereskia aculeata (barbados gooseberry)
Persea persea (alligator pear, avocado)
Phaseolus vulgaris and lunatus (bean and lima bean)
Psidium guayaba, montanum and cattleyana (guava)
Sapota zapotilla (sapodilla)
Smilax officinalis, papyracea and medica (sarsaparilla)
Solanum tuberosum, muricatum and melongena (potato, melon-pear and eggplant)
Theobroma cacao (chocolate tree)
Tropaeolum, majus and minus (nasturtium)
Vanilla planifolia, grandiflora and aromatica (vanilla)
Zamia integrifolia and furfuracea (Florida arrowroot, sago)
Zea mays (indian corn)

The foregoing lists contain some plants, such as Datura and poison ivy, that are poisonous, but were nevertheless taken internally and may have functioned as a source of vitamine. The Indians used Datura as an anesthetic in surgical operations and ate poison ivy in order to de-sensitize themselves. One might suppose that plants

that were considered to be medicines were taken in such small quantities, and so infrequently, that they could not have been important sources of vitamine. The quantities varied of course, but were sometimes considerable, three pints of a decoction often having been given in two days. Diarrhea was very prevalent among the Indians and hence medication was frequent.

The supply of wild plants was by no means unlimited and the Indians are supposed to have cultivated many species. Besides the corn, beans, squashes, pumpkins and potatoes that we have obtained from the Indians, they planted other species. The watermelon is generally supposed to have come from Africa but, according to Gilmore, the Indians of the Missouri River region cultivated a small spherical variety before the introduction of the modern varieties. Indians planted Jerusalem artichokes and sunflowers, and aided in the distribution of many wild fruits and land and water plants. Whether the Indians tilled the soil in the case of the latter seems doubtful, but they are said to have protected them. The Indians of the Pacific Coast did not cultivate the soil, but they lived largely on plant food. Their habit of gathering roots resulted in the name "Digger Indian."

From the standpoint of vitamines, the mode of preparation of food is of importance. The Indian method of boiling corn in potash or lime-water may have lessened the vitamine content in the corn but, since the boiling was applied to the whole grain, probably considerable water-soluble vitamine was left in it. Corn was eaten whole after this treatment or ground while wet. Mexicans grind wet corn on the metate, but some of them take the wet corn each day to an ixtamal mill and have it ground by power. Some Indians ground the corn in the dry state. Acorns were soaked in water after grinding, a process that probably removed much of the water-soluble vitamine. The great superiortiy of the Indian method of milling was due to the fact that the germ was not removed. The germ that contains the vitamine is removed from nearly all the cereals we eat, and fed to stock. This is good for the stock, but no one has been able to prove that it is good for us.

The antiscorbutic vitamine is the most perishable and difficult to store for winter. Drying of most vegetables destroys this vita-

mine. Only very acid foods, such as tomatoes or some fruits, have been shown to be antiscorbutic in the dry state. The Indian kept antiscorbutics for winter in the form of dried fruits and fresh foods. Not only were roots and tubers stored for winter, but such perishable things as watermelons were stored by wrapping in yucca leaves and suspending from the rafters.

The fact that Indians were well supplied with vitamine does not prove that vitamines are responsible for good teeth but suggests at least that the subject is worthy of further study. Many of the foods eaten by Indians required more mastication than ours. "Chewing" is considered bad manners by us and is discouraged in many ways. We have developed such a fear of germs that we discourage our children from "chewing" many raw plants that were "chewed" by Indians.

From consideration of the data compiled by Pickerill one might suppose that a good definition of a civilized man is a man with carious teeth. From this standpoint the Mexican is not completely civilized and we may have the opportunity of studying the process of his civilization. The difficulty in studying the question experimentally on animals is due to the fact that few animals are sufficiently civilized to develop caries, although old dogs frequently have pyorrhea.

III. SUMMARY

North American Indians had good teeth. This condition was probably due to complete adequacy of diet. The Indians ate a greater variety of foods than is generally supposed. Their vegetable foods were of such nature, and so preserved and prepared, as to furnish all three classes of vitamines. Since lack of vitamines causes changes in the teeth (scurvy) or improper development of the teeth (rickets), the abundance of vitamines in the diet of Indians probably had much to do with the state of their teeth. These are merely suggestions intended to stimulate research along this line.

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WILLIAM J. GIES

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PROMPT ANNOUNCEMENT AND CORRECTION OF A SERIOUS MISTAKE IN THE BINDING OF THE MARCH ISSUE OF THIS JOURNAL

WILLIAM J. GIES

By accident, pages 53 and 54 of this volume, in the paper by McClendon in the March issue, were omitted from that number when the copies were bound.

These two missing pages immediately follow this announcement They are appended to this issue, in place of the usual section on "proceedings of dental and stomatological societies," in order to isolate these pages conspicuously. We hope in this direct and special way not only effectively to announce and correct the oversight, but also to facilitate proper disposition of these two pages in the permanent binding of this volume.

We apologize for the error, and deeply regret the annoyance it has given, and will continue to give, to the readers of this JOURNAL.

Because of the omission from this issue of the section on "proceedings of dental and stomatological societies," that section in our issue for September will be correspondingly larger than usual.